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SELF-FERTILITY OF A CENTRAL OREGON SOURCE OF PONDEROSA PINE

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INTRODUCTION

This report will describe the effect of self-, cross-, and open- or wind-pollination on seed and seedling production of 19 ponderosa pine (*Pinus ponderosa* Laws.) trees in the eastern foothills of the Cascade Mountains south of Bend, Oreg. The study is part of a continuing investigation of self-fertility in several conifers growing in the Pacific Northwest to evaluate the effect of inbreeding on fertility and vigor and to provide information which may be useful in regeneration and tree improvement programs in these species.

MATERIALS AND METHODS

Study trees were located in two stands south of Bend, Oreg. One stand (Lava Butte) was at about 4,000-foot elevation and was pure ponderosa; the other (Kiwa Springs) was at about 4,500-foot elevation and was a mixture of ponderosa and lodgepole (*Pinus contorta* Dougl.) pines. Ten scattered trees were self- and cross-pollinated at each location.

Controlled pollinations using fresh pollen were made in the spring of 1967. Thirty to 50 female strobili on each tree were covered with isolation bags about 3 weeks prior to pollen shed. Approximately half of these strobili were self-pollinated and half cross-pollinated. Cross pollen was a mixture from six trees growing about 2 miles north of the Lava Butte group. The isolation bags were removed for 1 to 3 minutes while either self or cross pollen was poured directly on the female strobili.

Three weeks after pollination, isolation bags were replaced by cloth bags to protect conelets from insects. When bagged cones were collected in 1968, 10 to 25 open-pollinated cones were also collected from each of the trees. Number of female strobili were tallied when each of the operations was performed: May 25, 1967 (strobili just emerging), June 20 (pollination), July 15 (bagging to protect from insects), April 25, 1968 (replacement of torn insect bags), September 5 (cone collection).

Numbers of full-sized, normal-appearing seeds (hereafter called "round" seeds) were tallied for each cone. Round seeds were X-rayed and seeds with plump megagametophytes and embryos (hereafter called "filled" seeds) tallied. Filled seeds were further separated and recorded as to whether the embryo was greater than three-quarters the length of the embryo cavity, between one-half and three-quarters length, or less than one-half length.

Germination tests were conducted using seeds with full-sized embryos (i.e., seeds with embryos greater than three-quarters the length of the embryo cavity). Except for five families which had too few seeds, 100-seed lots were germinated in a commercial "germinator" at 30° C. day temperature and 20° C. night temperature with 12-hour photoperiods and thermoperiods. Prior to germination, seeds were stratified for 3 weeks at 5° C. The germination test was discontinued when no germination had occurred for a period of 1 week.

Separate germination tests were run on seeds with undersized embryos. The tests were the same as for seeds with full-sized embryos, except that stratification period was 6 weeks and the criterion of germination was a little different. For seeds with full-sized embryos, germination was considered complete when the radicle appeared. However, germination of seeds with undersized embryos was not

considered complete until some cotyledonary tissue could also be seen outside the seedcoat--these seeds frequently stop developing after only a small portion of the embryo protrudes.

Germinant seedlings from seeds with full-sized embryos were planted at 3- by 3-inch spacing in a nursery seedbed for growth and survival tests. The seedbed was under 45-percent shade and was watered and weeded regularly. Seedlings were arranged in "seed tree groups" with selfs in the center of each group and seedlings from cross- and wind-pollination of the same seed tree on either side of the selfs. Survival was recorded on 45 of the germinant seedlings (except for four families which did not have that many seedlings) at the end of the first growing season.

Differences between self- and cross-pollination or among self-, cross-, and wind-pollination were tested for significance using analysis of variance. All tests, except that for numbers of round seeds per cone, were based on percentage data, and these were angularly transformed before analysis.[1] Most analyses consisted only of effects due to seed tree and pollen treatment, which were tested against the interaction term. Two exceptions were the cone set analysis, where what appeared to be a large location difference was tested against variation among trees across pollen treatments within locations, and the analysis of relative self-fertility, where between locations mean square was tested against within locations mean square.

[1] Accompanying the conclusions of each statistical test are treatment means. These means are not the untransformed arc sine means, as one might suspect, but are the arithmetic means which are presented to allow readers to compare these averages with averages for other species.

RESULTS

Cone set.--Loss of conelets during first and second year was determined only for female strobili which had been self- and cross-pollinated; development of open-pollinated conelets was not recorded. Seventy-five percent of the strobili developed into collectible cones after cross-pollination; 70 percent, after self-pollination. This difference was not statistically significant. Table 1 gives cone development by area and type of pollination. Cone loss was significantly greater at Lava Butte than at Kiwa Springs, but no explanation is available. Percent cone loss or abortion was approximately the same during the first and second years of development.

Seed set.--Seed counts were classified into round seeds and filled seeds and were recorded for self-, cross-, and open-pollinated cones. Trees at the two areas were tested separately and together. In each test, differences between trees were significant, but there was no evidence for differences among pollen sources, which indicates that type of pollination (self, cross, or open) does not affect the production of round seeds. Averages are given in table 2.

Yield of filled seeds was expressed as filled seeds per round seeds x 100, because non-round or flat seed represented no potential for development into a filled seed with mature embryo. Since self- and cross-fertility were ultimately to be compared, it was desirable that they be compared using a base which would represent the maximum expression of fertility for each type of pollination and each tree.

Yield of filled seed was significantly reduced by selfing (table 3). Average yields after self-, cross-, and open-pollination were, respectively, 23.7, 66.5, and 75.2 filled seeds per 100 round seeds.

Table 1.--*Percent*[1] *healthy female strobili after self- and cross-pollination at beginning and end of second year of cone development*[2]

Location and number of trees	Beginning of year		End of year	
	Self	Cross	Self	Cross
Lava Butte (9)	71	84	57	61
Kiwa Springs (10)	93	93	82	89
All trees (19)	82	88	70	75

1/ Percent healthy female strobili at times given is based on number of strobili initially isolated for pollination.

2/ Effect of type of pollination on percent healthy strobili at time of collection (end of year) was tested with analysis of variance and was nonsignificant.

Table 2.--*Number of round seeds per cone after self-, cross-, and open-pollination*[1]

Location and number of trees	Type of pollination		
	Self	Cross	Open
Lava Butte (9)	92.5	92.2	85.0
Kiwa Springs (10)	110.6	113.7	106.7
All trees (19)	102.0	103.5	96.4

1/ Effect of type of pollen on number of round seeds per cone was tested by analysis of variance and found to be nonsignificant.

Table 3.--*Yield of filled seeds*[1/] *after self-, cross-, and open-pollination, and relative self-fertilities*

Tree number	Type of pollination and yield of filled seed			Relative self-fertility[2/]
	Self	Cross	Open	
	- - - - *Percent* - - - - -			
LB-2	2.0	52.1	65.7	0.038
LB-9	5.9	72.5	78.8	.081
LB-5	9.4	65.1	74.9	.144
LB-7	14.7	89.7	83.2	.164
KS-2	13.6	75.1	78.7	.181
LB-1	9.9	52.4	41.3	.189
KS-5	17.8	87.4	77.1	.204
KS-4	15.0	68.9	77.3	.218
LB-4	13.9	50.7	72.2	.274
KS-9	26.3	66.6	83.0	.395
KS-3	28.4	69.6	75.2	.408
KS-6	30.5	73.9	80.7	.413
LB-3	29.4	62.8	79.1	.468
KS-10	43.8	87.0	84.3	.503
KS-1	34.6	63.3	67.0	.547
KS-8	47.6	77.0	80.6	.618
LB-8	30.4	47.2	87.1	.644
KS-7	30.1	41.5	70.1	.725
LB-6	46.9	61.6	72.1	.761
Lava Butte average	18.1	61.6	72.7	[3/].307
Kiwa Springs average	28.8	71.0	77.4	[3/].421
Grand average	[4/5/]23.7	[4/5/]66.5	[4/5/]75.2	.367

[1/] Filled seed per round seed times 100.

[2/] Relative self-fertility is quotient of column 2 divided by column 3, that is, filled seed per round seed after selfing divided by filled seed per round seed after crossing.

[3/] Relative self-fertilities of trees from Lava Butte and Kiwa Springs were not significantly different.

[4/] Differences in yield of filled seeds among pollination types were significant at the 99-percent probability level when tested by analysis of variance.

[5/] Cones averaged just slightly more than 100 round seeds per cone, so average number of filled seeds per cone is about 23, 66, and 75 after self-, cross-, and open-pollination, respectively.

Differences among pollination types were significant; even the difference between cross- and open-pollination was significant at the 95-percent probability level. Why controlled cross-pollination should produce fewer filled seeds than wind-pollination is not known; however, higher seed yield after wind- than after cross-pollination has also been reported in other pine species.[2]

Relative self-fertility.--Relative self-fertility is defined as filled seeds per round seed after selfing divided by filled seeds per round seed following controlled crossing. Reasons for use of this ratio have been described elsewhere,[3] but basically it is to reduce variation which is not associated with the genotype of the embryo but which affects seed yield, such as applying pollen when conelets are not fully receptive. Relative self-fertilities of the 19 trees are arrayed in table 3.

Variation in relative self-fertility is large, e.g., one tree set about 4 percent as many seeds after selfing as after crossing, whereas another set 76 percent as many seeds after selfing as after crossing. Average self-fertility for the 19 trees is 35 to 40 percent (mean = 36.7; median tree = 39.5 percent). The average relative self-fertilities of Lava Butte trees and Kiwa Springs trees did not differ significantly.

Relationship between yield of filled seeds after self- and after cross- and wind-pollination.--This relationship was tested with the ponderosa pine data first by regressing yield of filled seeds per cone after cross- or open-pollination on yield of filled seeds per cone after self-pollination; and second, by making the same regressions on filled seed yields which were adjusted by treating filled seeds as a percent of round seeds per cone. This adjustment was made because cones from separate trees differed greatly in cone size and in round seed production (for example, a three-to-one difference in number of round seeds per cone for different trees). Regression coefficients are given in table 4. The regression without adjustment means that for a change of one filled seed per cone after selfing, an accompanying change of 0.40 filled seed per cone after crossing is predicted. The regression with adjustment means that for a change of 0.1 filled seed per round seed following selfing, an accompanying change of 0.004 filled seed per round seed after crossing is predicted.

Germination.-- Germination results are given in table 5 for the seeds with full-sized embryos. Germination percentages across all families were 95.1, 96.2, and 95.8, respectively, for seeds from self-, cross-, and open-pollination. These percentages were not statistically different, so there was no evidence that inbreeding adversely affected germination of seeds with full-sized embryos.

When germination of seeds with undersized embryos was included, however, selfed seeds gave slightly less germination than crossed seeds. This lesser germination was because seeds with full-sized embryos had higher germination than did seeds with undersized embryos, and seeds from cross- and open-pollination had a higher proportion of full-sized embryos than did seeds from self-pollination.

Germination percentages were 91.6, 85.4, and 91.6 (self-, cross-, and wind-pollinated seeds, respectively) for seeds with embryos one-half to three-quarters the length of the cavity, and were 39.7, 45.3, and 55.0, respectively, for seeds with embryos less than one-half the length of the embryo cavity.[4]

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[2] E. B. Snyder and A. E. Squillace. Cone and seed yields from controlled breeding of southern pines. Southern Forest Exp. Sta. USDA Forest Serv. Res. Pap. SO-22, 7 p. 1966.

[3] Frank C. Sorensen. Estimate of self-fertility in coastal Douglas-fir from inbreeding studies. (Submitted to Silvae Genetica, 1968.)

[4] Many of these seed lots were very small (five to 15 seeds), so differences were not analyzed statistically.

Table 4.--*Regression coefficients for yield of filled seeds after cross-pollination on yield after self-pollination, and for yield after wind-pollination on yield after self-pollination*

Type of pollination	Regression coefficients	
	Without adjustment[1/]	With adjustment[1/]
Self- and cross-pollination	0.40	0.014
Self- and wind-pollination	[2/] .84	.038

[1/] Adjustment for number of filled seeds per round seed.
[2/] Significant at the 95-percent probability level.

Seeds with embryos one-half to three-quarters the length of the embryo cavity made up 9.5, 2.8, and 4.0 percent of filled self-, cross-, and wind-pollinated seeds, respectively; seeds with embryos less than one-half the length of the embryo cavity made up 11.9, 2.9, and 4.5 percent of filled self-, cross-, and wind-pollinated seeds, respectively. Germination percentages, taking into account all types of filled seeds, were 88.2, 94.4, and 93.8, respectively, for self-, cross-, and wind-pollinated seeds.

First-year survival.--First-year survival was significantly less ($p < 0.05$) for the selfs than for the other two classes of seedlings (table 6). Although selfed families did not look quite as vigorous as noninbred progenies, the greater loss of selfed seedlings was not primarily due to any general weakness but to recessive lethal genes carried by four of the seed trees. Omitting mortality caused by these genes, survival was 97.3, 98.0, and 98.3 percent for progenies from self-, cross-, and open-pollination, respectively.

DISCUSSION

Although selfing in tree improvement may not have wide application,[5/] there may be cases where it is a useful technique.[6/] If so, self-fertility of ponderosa pine is high enough to permit the easy production of selfed seedlings. Selfing yielded about 37 percent as many filled seeds as crossing, and about 32 percent as many 1-year-old seedlings.

Parents that produce high seed yields when self-pollinated may tend to produce high yields when wind-pollinated (see footnote 5). A slight relationship of this type held for ponderosa pine; however, it appeared that the relationship occurred

[5] E. B. Snyder. Seed yield and nursery performance of self-pollinated slash pines. Forest Sci. 14: 69-74. 1968.

[6] A. L. Orr-Ewing. Inbreeding to the S_2 generation in Douglas-fir. Second World Consultation on Forest Tree Breeding, Sect. III (8/6), 13 p. 1969.

Table 5.--*Percent germination for self-, cross-, and open-pollinated seeds*[1/]

Seed tree	Pollen source		
	Self	Cross	Open
LB-1	84	95	88
LB-3	100	97	96
LB-4	98	99	100
LB-5	79	95	100
LB-6	100	100	100
LB-7	100	100	93
LB-8	98	93	100
LB-9	97	99	98
KS-1	100	99	97
KS-2	92	95	97
KS-3	87	90	91
KS-4	99	99	98
KS-5	98	100	100
KS-6	95	95	93
KS-7	99	98	88
KS-8	92	86	89
KS-9	97	94	97
KS-10	97	98	100
Average	95.1	96.2	95.8

[1/] Only filled seeds with embryos greater than three-fourths the length of the embryo cavity were used in this test.

Based on 100 filled seeds per progeny except for LB-1 x self, LB-4 x self, LB-9 x self, KS-3 x self, and KS-3 x wind, which had 43, 54, 79, 15, and 90 seeds, respectively.

Differences in percent germination among self-, cross-, and open-pollinated seeds were not statistically significant.

primarily because of differences among trees in cone size and round seed production. Still, it should be pointed out that even after adjustment for cone size, there was a small tendency for trees with greater self-fertility to produce a few more filled seeds when open-pollinated than did trees with lower self-fertility. If this relationship is real, it probably reflects a tendency for relatively self-fertile trees to produce a slightly higher proportion of selfed seeds in their wind-pollinated progenies.

Various pine species show considerable diversity in the effect of self-pollination on seed traits, with some species being more and some less self-fertile than ponderosa pine.[7/] However, compared with Douglas-fir, the one other western conifer whose average relative self-fertility has

[7] E. C. Franklin. Artificial self-pollination and natural inbreeding in *Pinus taeda* L. 127 p. 1968. (Ph.D. thesis on file at North Carolina State Univ., Raleigh.) Appendix table 3.

Table 6.--*Survival at end of first growing season of ponderosa pine seedlings*[1/] *from self-, cross-, and wind-pollination*[2/]

(In percent)

Seed tree	Pollen type		
	Self	Cross	Wind
LB-1	100	96	96
LB-3	98	100	100
LB-4[3/]	69(98)	100	98(100)
LB-5	98	100	100
LB-6	96	98	100
LB-7	96	96	91
LB-8[3/]	62(82)	98	98(100)
LB-9	96	100	100
KS-1	93	91	98
KS-2	96	96	98
KS-3	100	100	93
KS-4	100	98	100
KS-5	100	100	100
KS-6[3/]	60(100)	100	98
KS-7	100	96	98
KS-8[3/]	62(100)	98	98(100)
KS-9	100	100	98
KS-10	98	98	100
Average	90.2	98.0	98.0

1/ Based on 45 established seedlings in each family except for families KS-3 x self, cross, and wind, and LB-1 x self, which started with 12, 15, 15, and 33 seedlings, respectively.

2/ Differences in first-year survival were significant at the 95-percent probability level.

3/ These seed trees all carried recessive seedling lethal factors, whose effect was expressed soon after the seedcoat was shed. Number in parentheses is survival when mortality caused by these specific lethal alleles was omitted.

been studied and which is estimated at 10 to 12 percent (see footnote 3), ponderosa pine is considerably more self-fertile. In Douglas-fir, except for a small percentage of relatively self-fertile trees, the usual and main effect of natural selfing should be to decrease seed set. But this is not necessarily so with ponderosa pine. Greater self-fertility could lead to a moderately high incidence of selfed seedlings in an open-pollinated population. If this is accompanied by inbreeding depression, then *natural* self-pollination could lead to a decrease in seedling vigor as well as a decrease in seed set.

In other pine species, it has been observed that the incidence of natural selfing is higher in the lower than in the upper part of the crown (see footnote 7).[8] This also can be reasonably assumed to be true for ponderosa pine, and if so, it has implications for seed collection practices. If self-fertility were low, then the collector would only get a higher proportion of empty seeds in a collection from the lower crown. But if self-fertility is relatively high, as is indicated here for ponderosa pine in Oregon, then a collection from the lower crown will not only yield somewhat fewer seeds but will also include seeds with a higher level of inbreeding, which most likely will produce seedlings with reduced vigor. It will probably be some time before there are reliable figures to attach to the amount of natural self-pollination and to the amount by which inbreeding reduces vigor. But for the present, it appears that good management practices dictate upper crown seed collections whenever possible.

[8] D. P. Fowler. Natural self-fertilization in three jack pines and its implications in seed orchard management. Forest Sci. 11: 55-58. 1965.

SUMMARY

Yield of filled seeds after self-, cross-, and wind-pollination was determined on 19 ponderosa pine trees at two locations in central Oregon. Type of pollination had no statistically significant effect on cone set or production of round seeds. However, production of filled seeds (that is, relative self-fertility) was only about 35 to 40 percent as great after selfing as after crossing. Relative self-fertility among the trees varied from 4 percent to 76 percent. Germination percent of filled seeds and first-year seedling survival were slightly less for inbred progenies than for cross-bred and open-pollinated progenies.

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Sorensen, Frank C.
1970. Self-fertility of a central Oregon source of ponderosa pine. USDA Forest Serv. Res. Pap. PNW-109, 9 p. Pacific Northwest Forest and Range Experiment Station, Portland, Oregon.

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